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14. ABSTRACT

Wnt proteins form a family of highly conserved secreted signaling molecules that regulate cell-cell interactions. Deregulation of Wnt signaling plays a role in breast cancer. Thus, proteins that regulate the activities of Wnt signaling might have a significant impact on oncogenesis. We discovered a novel nuclear protein that antagonizes β-catenin-mediated transcriptional activation in mammalian cultured cells. Mechanistically, Chibby competes with TCF to bind to β-catenin. Moreover, genetic experiments with the *Drosophila* Chibby gene indicates that Chibby acts downstream of *Drosophila* Wnt-1 and upstream of β-catenin. Taken together, Chibby represents a conserved nuclear inhibitor of Wnt signaling. The hypothesis of this project was that mutation or alteration of Chibby might lead to hyper-active Wnt signaling and enhance the turmorigenicity or progression of breast cancer. To test this hypothesis, we surveyed a large number of breast cancer samples for mutation or silenicing of the Chibby locus. No changes from normal were detected. Thus, our hypothesis may have been incorrect or that loss of Chibby is a rather rare event in breast cancer.

15. SUBJECT TERMS
Beta-catenin, chibby, wnts, signal transduction, breast cancer

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Introduction

Wnt proteins form a family of highly conserved secreted signaling molecules that regulate cell-cell interactions. The Wnt signaling pathway plays various roles during breast development and in response to systemic hormones[1]. Activation of the Wnt signaling pathway leads to stabilization of the protein β-catenin. β-catenin consequently transports into the nucleus, binds the transcription factor TCF, and activates transcription of target genes including cyclin D1 and c-myc[2]. Deregulation of Wnt signaling plays a role in breast cancer. Wnt genes are overexpressed in some human breast cancers, and there is growing evidence that downstream components of the Wnt pathway are activated in a significant proportion of breast tumors[3]. Changes in β-catenin abundance are observed in a majority of human breast tumors, and elevated levels of β-catenin in breast adenocarcinomas are associated with poor prognosis for recovery[4]. Thus, proteins that regulate the nuclear activities of β-catenin might have a significant impact on oncogenesis. Recently, we discovered a novel nuclear protein that associates with β-catenin[5]. Chibby antagonizes β-catenin-mediated transcriptional activation in mammalian cultured cells. Mechanistically, Chibby competes with TCF to bind to β-catenin. Moreover, genetic experiments with the *Drosophila* Chibby gene indicates that Chibby acts downstream of *Drosophila* Wnt-1 and upstream of β-catenin[5]. Taken together, Chibby represents a conserved nuclear inhibitor of β-catenin signaling in both vertebrates and *Drosophila*. Since elevated β-catenin signaling has been reported in breast cancer, then decreased levels of Chibby expression might be relevant to tumor progression, raising the possibility that Chibby is a tumor suppressor gene.

Body

The research was carried out by Shinji Yamaguchi, Ph.D. Dr. Yamaguchi was a postdoctoral fellow in my lab, and was a co-discoverer of the Chibby gene. He was highly qualified to carry out the work, and did so in effective fashion. He was supported by this grant for its term.

Task 1. Generation of anti-Chibby antibody

The human Chibby coding sequence was PCR amplified and subcloned into a pET bacterial expression vector. The plasmid was transformed into *E. coli*, and expression of Chibby protein was induced. Virtually all protein was insoluble and pelleted into inclusion bodies. The inclusion bodies were purified and were determined to be about 95% Chibby protein. This protein was solubilized by guanidinium hydrochloride and renatured in vitro. After dialysis, soluble protein was used to immunize rabbits. Immune sera were collected after two-three boosters and tested for immunoreactivity with recombinant Chibby protein by Western blot. The sera tested positive for anti-Chibby activity and was further tested. Extract from human cell lines were run on a Western blot and reacted with the anti-Chibby sera. While the sera recognized a protein of the predicted size of Chibby, the signal was weak and background was high. Affinity purification of the antibody did not improve the weak signal on Western blots. Thus the antibody was of limited utility.

Task 2. Extensive evaluation of Chibby protein abundance in breast tumor samples

We obtained a limited number of normal and breast cancer cell lines from the ATCC collection. We also obtained an extensive number of breast cancer lines from Dr. Vimla Band at the Evanston Northwestern Healthcare Institute. Primary tissue samples were also obtained in blind fashion. A total of 35 different samples were collected. These were all extracted for total RNA or DNA. DNA

samples were incubated with primers to PCR amplify the Chibby genomic region (coding sequence). These PCR products were sequenced to determine if any mutations in the Chibby gene are present in any samples. Our control was DNA from several non-cancerous human lines. Sequence analysis determined that none of the breast cancer samples contained any sequence rearrangements.

RNA samples were selected for polyA+ mRNA by affinity purification, and samples were run on Northern blots. These were probed with human Chibby antisense RNA to detect the presence of Chibby mRNA. In control samples, a single mRNA species of the predicted size for human Chibby was detected. In all cancer samples, the same species was detected at comparable abundance and size. Thus, no perturbations of Chibby mRNA expression were detected in the various breast cancer samples. We also performed initial surveys of Chibby protein with the anti-Chibby antibdy but the quality of the results was not sufficient to warrant a complete survey.

Task 3. Transfection of cell lines exhibiting depleted Chibby levels
This task was dependent upon our discovery of one or more breast cancer cell lines that had mutated Chibby coding sequence or were not expressing the gene at normal levels. Unfortunately, all cancer lines that we tested did not reveal such deficits. Thus, we did not complete this task. However, we performed the needed pilot experiments in which human Chibby was subcloned into a CMV expression vector. This vector was transfected into control cell line (Hela) and examined for expression. We found that Chibby was effectively expressed from the vector. We also cotransfected the TOPFLASH reporter plasmid with Chibby into Hela cells, but observed no effect on TOPFLASH expression.

Key Research Accomplishments

- Production of anti-human-Chibby antibody
- Survey of 35 breast cancer samples for mutations or alterations in the Chibby locus
- Overexpression of human Chibby in cell culture

Reportable Outcomes

The results of this research were presented by Dr. Yamaguchi at a meeting for the Japan Society for the Promotion of Science held in Tokyo in 2004.

Dr. Yamaguchi received an appointment to the faculty at University of Teikya in Japan as an Assistant Professor, based on the experience and training he received from this grant. He is extremely grateful for the support given to him by this grant while in my lab.

Conclusions

Our hypothesis at the beginning of this grant was that Chibby might be a tumor suppressor gene. Based on what we knew about it from our research on animal models, it seemed to be a very reasonable hypothesis. That is, mutation of Chibby could cause hyper-activation of Wnt signaling, which would lead to cell growth even when cells should not grow. It is well established that hyper-activation of Wnt signaling is a causative effect in breast cancer, and it seemed logical that breast

cancers might be stimulated by loss of Chibby. We tested the hypthesis by surveying many breast cancer samples for mutation or alteration of Chibby, and found none. Thus, our hypothesis appears to have been incorrect. Nevertheless, it was important to test it, because if we had found Chibby was a tumor suppressor, it would have offered a novel treatment course for breast cancer. The knowledge gained from our study is more relevant as a scientific than medical product.

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